Our results support a simple satisfactory explanation for the presence of the non-heterocystous cyanobacteria *Trichodesmium* spp. in the (sub) tropical oceans and their absence in temperate and cold seas as well as in freshwater and brackish environments. Although we have also provided an explanation for the exclusion of free-living heterocystous cyanobacteria in the nitrogen-depleted euphotic zone of the pelagic tropical ocean, our findings do not explain why these organisms are virtually absent from temperate and polar marine waters. It seems that other factors prevent the proliferation of N_2-fixing cyanobacteria in these environments. We can speculate about possible candidates such as the availability of iron or phosphorus, but a sound explanation cannot be offered at present. □

Methods

Strains

Nodularia spumigena (strain CCY 9414) and *Anabaena* sp. (strain CCY 9901) were grown at 20 °C in medium free of combined nitrogen, comprising one part ASN3 and two parts BG 11 (ref. 17), giving a salinity of 11. *Nodularia spumigena* was also grown at 25 °C. For comparison, we measured nitrogenase activity in 19 other heterocystous cyanobacteria (see Supplementary Information). *Trichodesmium* sp. (IMS101) was grown at 25 °C in YBC II medium[18].

Nitrogenase activity and irradiance curves

Nitrogenase activity was measured by an on-line acetylene reduction assay equipped with a gas chromatograph (GC-FID)[19]. Nitrogenase versus irradiance curves were determined with the cyanobacteria immobilized on glass fibre filters (Whatman) in a temperature-controlled incubator[19]. For each temperature at which a measurement was made, nitrogenase versus irradiance curves were recorded at 20% O_2. A slide projector with a set of ten neutral density filters (Balzers) provided photon irradiances from 0 to 1,600 μmol m^{-2} s^{-1}. Light response curves were fitted in Origin 6.0 (Microcal) by nonlinear least of squares fitting, using the Levenberg–Marquardt algorithm with the rectangular hyperbola model[12,20]

$$N_m\left(\frac{\alpha I}{N_m+\alpha I}\right)+N_d$$

where N_m is the nitrogenase activity at saturating irradiances minus N_d, α is the light affinity coefficient for nitrogenase activity, and I is the photon irradiance. N_{tot} was calculated by summing N_d and N_m (ref. 12).

Other parameters

The O_2 optima of acetylene reduction assay at the different incubation temperatures were determined by measuring N_2 fixation at different O_2 concentrations (0, 2.5, 5, 10, 20 and 30% O_2) at each photon irradiance. The concentration of O_2 and the diffusion coefficients used in equations (1) and (2) were taken from the tables of Seawater and Gases (http://www.unisense.com/support/pdf/gas_tables.pdf). The model parameters for the boundary layer thickness and cell diameter were arbitrary values. We tested the robustness of the model towards these parameters. Changing their values did not alter the relative fluxes at the different salinities and temperatures.

We calculated Q_{10}* by fitting the temperature response curves with the equation $Y = Y_0\exp(\lambda T)$, where $Q_{10}^* = \exp(\lambda 10)$. The Q_{10}* for *Trichodesmium* IMS101 at 15–20 °C and for *N. spumigena* at 10-15 °C was estimated with a linear function, because an exponential fit through two points may be a source of erroneous results.

Chlorophyll *a* was extracted with 96% ethanol for 24 h in the dark. Absorption was read at 665 nm with a spectrophotometer, and the chlorophyll concentration was calculated by using an absorption coefficient of 72.3 ml mg^{-1} cm^{-1}.

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Correspondence and requests for materials should be addressed to M.S. (M.Staal@nioo.knaw.nl).

Jelly belly protein activates the receptor tyrosine kinase Alk to specify visceral muscle pioneers

Hsiu-Hsiang Lee[1], Audra Norris[2], Joseph B. Weiss[2]* & Manfred Frasch[1]*

[1]*Brookdale Department of Molecular, Cell and Developmental Biology, Mount Sinai School of Medicine, One Gustave L. Levy Place, Box 1020, New York, New York 10029, USA*
[2]*Molecular Medicine and Cardiology, Oregon Health and Sciences University, 3181 SW Sam Jackson Park Road/NRC3, Portland, Oregon 97201-1807, USA*

* These authors contributed equally to this work

The secreted protein Jelly belly (Jeb) is required for an essential signalling event in *Drosophila* muscle development. In the absence of functional Jeb, visceral muscle precursors are normally specified but fail to migrate and differentiate[1]. The structure and distribution of Jeb protein implies that Jeb functions as a signal to organize the development of visceral muscles[1]. Here we show that the Jeb receptor is the *Drosophila* homologue of anaplastic lymphoma kinase (Alk), a receptor tyrosine kinase of the insulin receptor superfamily. Human ALK was originally identified as a proto-oncogene, but its normal function in mammals is not known[2]. In *Drosophila*, localized Jeb activates Alk and the downstream Ras/mitogen-activated protein kinase cascade to specify a select group of visceral muscle precursors as muscle-patterning pioneers. Jeb/Alk signalling induces the myoblast fusion gene *dumbfounded* (*duf*; also known as *kirre*) as well as *org-1*, a *Drosophila* homologue of mammalian *TBX1*, in these cells.

Signalling molecules and their receptors orchestrate cell fate decisions essential to organogenesis. Studies of mesoderm development in *Drosophila* have highlighted the role of evolutionarily conserved signalling systems, and the transcription factors they regulate, in the elaboration of the mesoderm into its derivative tissues. The earliest cell fate assignments in the mesoderm are coordinated by inductive signals from the ectoderm. Decapentaplegic (Dpp), a *Drosophila* BMP signal, induces subjacent dorsal

mesoderm to express Tinman (Tin), a homeodomain protein essential for heart, visceral and dorsal somatic mesoderm development[3–5]. Dpp and Tin, together with Hedgehog, induce visceral mesoderm by activating the expression of two transcription factors, Bagpipe (Bap) and Biniou (Bin)[5–8]. A third signal, Wingless, antagonizes these visceral mesoderm-inducing activities[7,9,10]. The combined actions of ectodermally derived Dpp, Hedgehog and Wingless generate segmental clusters of visceral mesoderm precursors in the dorsal mesoderm.

The secreted protein Jeb is necessary for the subsequent rearrangement of these segmental clusters of visceral mesoderm precursors into bilateral longitudinal bands and for visceral muscle differentiation[1]. Jeb is produced in ventral somatic mesoderm, locally secreted, and is specifically taken up by the visceral mesoderm cells[1]. Its detailed developmental role, however, has not been defined. We show that one critical function of Jeb signalling is to subdivide the pool of visceral mesoderm precursors into two distinct subtypes: muscle founders and fusion-competent cells (Fig. 1). This subdivision is key to the muscle specification and fusion pathway, a hierarchical system for patterning muscles (reviewed in refs 11, 12). As first shown for somatic muscle development in *Drosophila*, founder myoblasts are patterning pioneers[13,14]. They establish specific muscles and recruit fusion-competent myoblasts to fuse with them into mature syncytial muscle fibres. Founder myoblasts and fusion-competent myoblasts are identified by the expression of functional components of the myoblast fusion pathway. Founder cells express Duf, a transmembrane protein necessary for recruitment of fusion-competent cells. Fusion-competent cells express Sticks and stones (Sns), a transmembrane protein also required for fusion.

Besides *duf*[15,16], we have found that the expression of a *Drosophila* homologue of mammalian TBX1—*org-1* (ref. 17), initially detectable in all visceral mesoderm precursors (Fig. 1a)—is rapidly restricted to founder cells (Fig. 1b, c). TBX1, a T-box transcription factor, is required for cardiovascular development, and in humans haploinsufficiency of its function results in the congenital cardiovascular abnormalities associated with 22q deletions in DiGeorge/velocardiofacial syndrome (reviewed in ref. 18). As shown in Fig. 1, the expression of both *org-1* and *duf* markedly responds to Jeb. Both are absent from the visceral mesoderm in stage 11–12 *jeb* mutant embryos (Fig. 1e, h), whereas ectopic expression of Jeb activates them throughout the visceral mesoderm (Fig. 1f, i). A third gene, *sns*, normally expressed in fusion-competent cells in a pattern reciprocal to *duf* is negatively regulated by Jeb (see Supplementary Fig. 1a–c).

Positive regulation of *duf* and negative regulation of *sns* implies that Jeb signalling specifies visceral mesoderm founders. As assayed by the markers *duf*, *org-1* and *sns*, no visceral muscle founders are specified in *jeb* mutant embryos. Instead all visceral mesoderm precursors become fusion-competent myoblasts. The consequence of absent visceral mesoderm founders, as shown by cell-lineage experiments (Fig. 1j, k; see also Supplementary Fig. 1d, e), is fusion of visceral fusion-competent myoblasts with somatic muscle founders and loss of visceral musculature. Somatic muscle patterning, however, is unaffected[1].

Localized activation of the Ras/mitogen-activated protein kinase (MAPK) cascade in the visceral mesoderm has been noted previously[19]. In the somatic muscle lineage this pathway is required for founder cell specification[20]. We therefore hypothesized that Jeb signals through the Ras/MAPK cascade in the visceral mesoderm. As shown in Figs 2a and 3a, b, activated MAPK is indeed detected in the visceral mesoderm precursors that take up Jeb. The observed overlapping signals for diphospho-MAPK and *org-1*, as well as the exclusive staining patterns for diphospho-MAPK and *sns* (Fig. 2b and data not shown), confirm that the MAPK pathway is activated in presumptive visceral muscle founders. Moreover, Jeb signalling is necessary and sufficient to activate the Ras/MAPK cascade in visceral mesoderm precursors. Immunostaining of *jeb* mutant

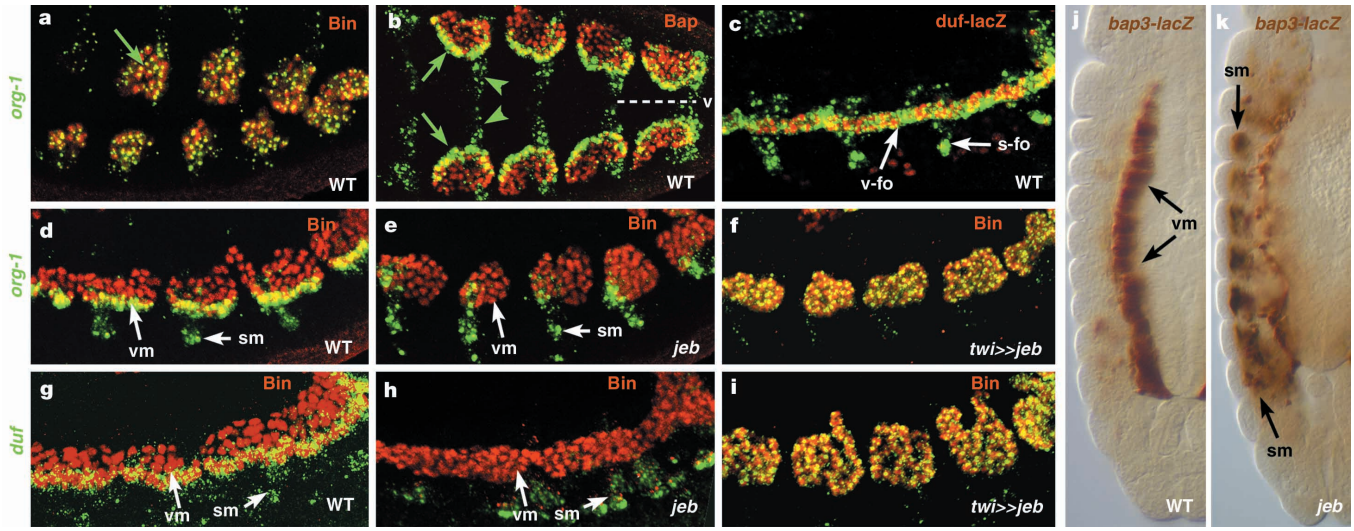

Figure 1 *jeb* specifies founder cell fates in the visceral mesoderm. Visceral mesoderm nuclei are marked by Bin or Bap protein staining (red), as indicated. mRNA probes are shown in green. **a**, Stage 9 embryo showing *org-1* mRNA (arrow) in all cells of the visceral mesoderm primordia. **b**, Ventral view of a stage 11 embryo. *org-1* expression (arrows) is restricted to the ventral row of cells in each visceral mesoderm cell cluster (arrowheads: somatic mesodermal *org-1*). v, ventral. **c**–**i**, Lateral high magnifications. **c**, At mid-stage 12, after a round of mitotic divisions of the progenitors, *org-1* mRNA (green) and *duf-lacZ* (*rP298* enhancer trap; red) are co-expressed in the two rows of visceral muscle founders. v-fo, visceral muscle founder; s-fo, somatic muscle founder. **d**, Stage 11 wild-type embryo showing *org-1* in muscle progenitors of the visceral mesoderm (vm). sm, somatic mesoderm. **e**, Stage 11 *jeb* mutant embryo lacking *org-1* expression in the visceral mesoderm primordia. **f**, Stage 11 embryo; ectopic *jeb* expression in the early mesoderm results in ectopic *org-1* expression in all visceral mesoderm cells. **g**, Stage 12 wild-type embryo showing *duf* expression in visceral muscle founders. **h**, Stage 12 *jeb* mutant embryo showing lack of *duf* expression in the visceral mesoderm. **i**, Stage 12 embryo with *twi*-driven ectopic *jeb*, which results in *duf* expression in all cells of the visceral mesoderm primordia. **j**, Stage 13 *bap3-lacZ* wild-type embryo (ventral/left side) showing βGal in the visceral mesoderm. **k**, Analogous *jeb* mutant embryo carrying *bap3-lacZ* shows βGal predominantly in somatic muscle syncytia.

embryos demonstrates absent diphospho-MAPK in the ventral visceral mesoderm cells that normally accumulate Jeb and become founders (Fig. 2c). As with founder cell markers, ectopic Jeb produces ectopic diphospho-MAPK, but only in the visceral mesoderm (Fig. 2d).

The expanded expression of *org-1* upon mesodermal expression of activated versions of *Drosophila* Ras (Fig. 2e) and human Raf (Fig. 2f) implicates the Ras pathway in MAPK activation and founder cell specification in the visceral mesoderm. If Jeb signals through the Ras/MAPK pathway, then activation of this pathway should rescue *jeb* mutations. As shown in Fig. 2g, h, this prediction is true. As judged by expression of fasciclin III, a marker of visceral mesoderm differentiation, expression of activated Ras can substantially rescue *jeb* mutant embryos.

The observed effects of ectopic Jeb are limited to the visceral mesoderm. Together with the observation that uptake of Jeb into visceral mesoderm cells requires *shibire*-mediated endocytosis[1], these data imply that Jeb acts through a tissue-specific receptor, which is coupled to the Ras/MAPK pathway. The receptor tyrosine kinase *Drosophila Alk*, a homologue of the human proto-oncogene anaplastic lymphoma kinase (ALK), is expressed in the early visceral mesoderm[21]. We therefore hypothesized that *Drosophila* Alk is the Jeb receptor. As shown in Fig. 3a, b, *Alk* messenger RNA is expressed in all cells of the trunk visceral mesoderm directly adjacent to the Jeb-expressing cells. In visceral mesoderm cells that both express Alk and take up Jeb[1] we detect diphospho-MAPK (Fig. 3a, b).

We tested whether, similar to Jeb, Alk activity is required for the specification of visceral mesoderm founder cells. As shown in Fig. 3d, embryos homozygous for a deficiency uncovering the *Alk* locus lack *org-1* expression in presumptive visceral mesoderm founders, a phenotype that can be rescued by expressing an *Alk* minigene in visceral mesoderm precursors (see Supplementary Fig. 2a, b). Mesodermal expression of a kinase-deficient, dominant interfering form of Alk produces an identical phenotype (Fig. 3e).

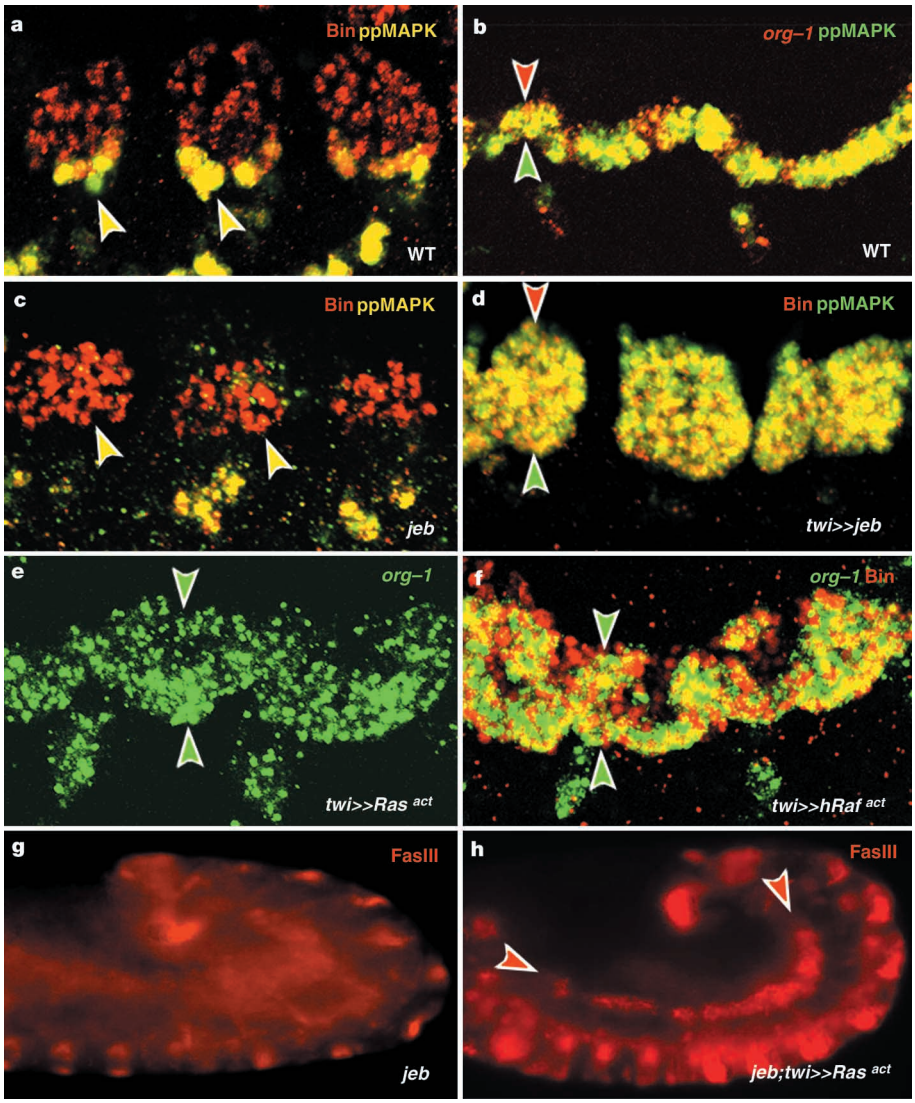

Figure 2 *jeb*-dependent Ras/MAPK signalling triggers founder cell specification in the visceral mesoderm. **a**–**f**, Lateral high magnifications. **a**, Anti-diphospho-MAPK (ppMAPK) antibodies detect activated MAPK (yellow, arrowheads) in the ventral cell rows of the visceral mesoderm primordia (red) at stage 10. **b**, Co-localization of ppMAPK (green, arrowhead) and *org-1* mRNA (red, arrowhead) at stage 11 identify cells with activated MAPK as visceral muscle founders. **c**, *jeb* mutant embryo lacking activated MAPK (yellow, arrowheads) in the visceral mesoderm primordia (red). **d**, Stage 11 embryo with *twi*-driven ectopic *jeb* expression. All cells of the visceral mesoderm primordia (red, arrowhead) contain activated MAPK (green, arrowhead). **e**, **f**, Early stage 12 embryos with ectopic expression of activated Ras1 and activated human Raf showing strongly expanded *org-1* expression (green, arrowheads) in the visceral mesoderm. **g**, Stage 12 *jeb* mutant embryo showing loss of fasciclin III expression in the visceral mesoderm. **h**, Stage 12 *jeb* mutant embryo with ectopic expression of activated Ras1 as in **e**. Fasciclin III expression in the visceral mesoderm is rescued (arrowheads).

RNA-mediated interference (RNAi) injection experiments further confirm that *Alk* is specifically required for visceral mesoderm founder specification. βGal staining of *bap3-lacZ* embryos injected with double-stranded (ds)*Alk* RNA demonstrates transformation of visceral into somatic muscle fates (Fig. 3f). Furthermore, injection of ds*Alk* RNA into *duf-lacZ* embryos results in strongly reduced or absent expression of this founder cell marker in the visceral mesoderm (Fig. 3h; see control in Fig. 3g). These RNAi phenotypes resemble the phenotypes of *jeb* mutant embryos, although they are less severe (see Fig. 1h, k).

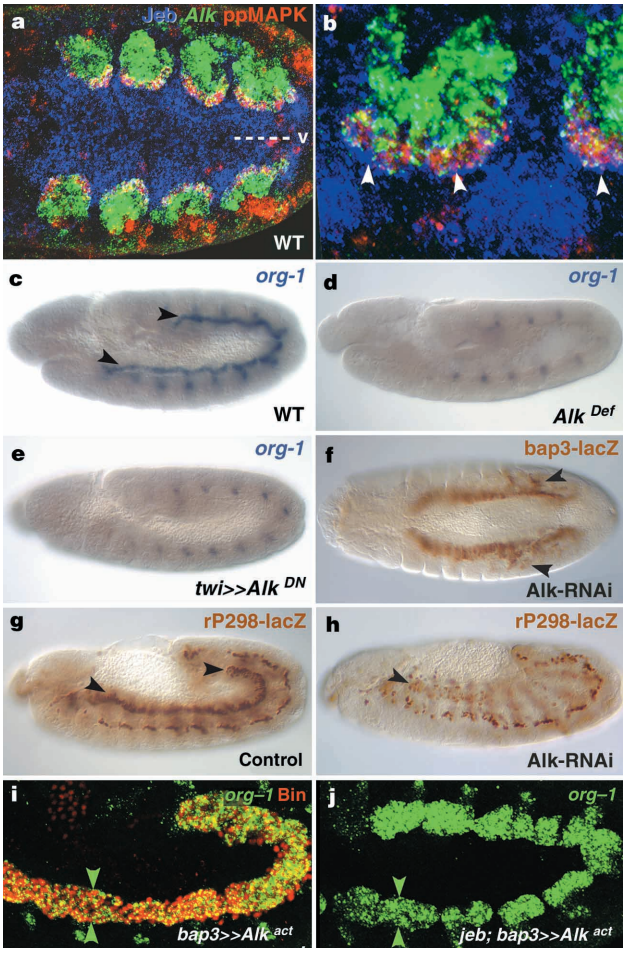

Figure 3 Jeb acts through the receptor tyrosine kinase Alk to specify visceral muscle founder cells. **a**, **b**, Ventral view (**a**) and high-magnification view of the lateral mesoderm (**b**) of stage 10 embryos triply stained for Jeb protein (blue), *Alk* mRNA (green) and activated MAPK (red). Jeb is predominantly detected in the ventrolateral somatic mesoderm and diffused Jeb is enriched along the visceral muscle progenitors (arrowheads), which contain activated MAPK. **c**, *org-1* mRNA expression in visceral (arrowheads) and somatic muscle progenitors of a stage 11 wild-type embryo. **d**, In a stage 11 embryo homozygous for *Df(2R)vg89e88* (Alk^{Def}) *org-1* expression in the visceral mesoderm is missing. **e**, Stage 11 embryo with ectopic expression of dominant-negative Alk lacking *org-1* expression in the visceral mesoderm. **f**, Late stage 12 *bap3-lacZ* embryo injected with ds*Alk* RNA. Near the posterior injection site, visceral mesoderm cells have assumed somatic mesodermal fates (arrowheads). **g**, Stage 12 *rP298* control embryo showing *duf* enhancer-driven βGal in visceral (arrowheads) and somatic muscle founders. **h**, Stage 12 *rP298* embryo injected with ds*Alk* RNA posteriorly. Except for traces at the anterior (arrowhead), *duf* enhancer-driven βGal is missing in the visceral mesoderm, but is unaffected in the somatic mesoderm. **i**, Stage 12 embryo with ectopic expression of activated Alk in the visceral mesoderm. *org-1* mRNA expression (green, arrowheads) has expanded into all cells of the trunk visceral mesoderm (red). **j**, Stage 11 *jeb* mutant embryo with ectopic expression of activated Alk in the visceral mesoderm. *org-1* mRNA expression in the visceral mesoderm (arrowheads) is rescued and expanded.

The loss of *duf* expression and expansion of *sns* expression in the visceral mesoderm on expression of dominant-negative Alk (see Supplementary Fig. 2c, d) is identical to a *jeb* null mutant phenotype as well. Conversely, the expansion of *org-1* expression in the visceral mesoderm on expression of activated Alk (a fusion protein analogous to the human oncogenic version, NPM-ALK[22]) is indistinguishable from the effects of expression of ectopic Jeb, activated Ras and activated Raf (Fig. 3i; compare with Figs 1f, 2e and f, respectively). Finally, forced expression of activated Alk in homozygous *jeb* mutant backgrounds is able to rescue (and compared with wild type expand) *org-1* expression in the visceral mesoderm (Fig. 3j) and to restore midgut morphogenesis (see Supplementary Fig. 2e–g).

To confirm that Jeb signals through Alk we have determined that Jeb binds Alk with high affinity, and that Jeb binding to Alk activates the Ras/MAP kinase cascade. In these experiments we used Jeb–alkaline phosphatase fusion proteins (Jeb–AP)[23]. To establish qualitatively the binding of Jeb to Alk, we visualized the specific association of Jeb–AP with *Alk*-transfected mammalian tissue culture cells. As shown in Fig. 4a, *Alk*-transfected cells bind Jeb–AP. By contrast, Alk-transfected cells do not bind either an equivalent concentration of alkaline phosphatase alone or a Jeb–AP fusion protein that lacks the type-A LDL receptor repeat in Jeb. This truncated version of Jeb resembles a mutant protein encoded by a null allele of *jeb*. The truncated protein does not accumulate in visceral mesoderm cells[1]. Binding of Jeb depends on Alk, as demonstrated with non-transfected cells that were incubated with full-length Jeb–AP (data not shown).

We used a similar assay to demonstrate that the Jeb–Alk interaction is specific and has high affinity. As shown in Fig. 4b, Jeb binding to *Alk*-transfected cells is saturable at nanomolar concentrations. Scatchard analysis demonstrates a single class of high-affinity Jeb-binding site with a dissociation constant (K_d) of 2.2 nM (Fig. 4c). No binding was observed with either alkaline phosphatase alone or Jeb–AP that lacks the type-A LDL receptor repeat. We also confirmed Jeb-dependent activation of the Ras/MAP kinase cascade in this system (Fig. 4d). The concentration dependence of Ras/MAP kinase activation by Jeb correlates well with our binding data. Approximately half-maximal activation occurs in the range of 2–3 nM. As *in vivo*, removing the type-A LDL receptor repeat from Jeb abrogates Ras/MAP kinase activation (Fig. 4e).

We have shown that Jeb activates the Ras/MAPK cascade both *in vivo* and in *Alk*-transfected tissue culture cells. Jeb binds Alk with high affinity. *In vivo* Jeb accumulates in visceral muscle founder cells and, in late-stage embryos, in axons of the central nervous system. These patterns of Jeb accumulation are absent from *Alk*-deficient embryos (data not shown; see Supplementary Fig. 3) and in *jeb* mutants that produce an Alk-binding-deficient version of Jeb[1]. Biochemical and genetic interference with Alk function produces phenotypes identical to *jeb* mutations. We have also shown that a critical function of Jeb signalling is to specify visceral muscle founder cells—patterning pioneers essential to midgut morphogenesis. Structurally Jeb belongs to a class of signalling molecules with type-A LDL receptor repeats as one of their functional domains. Others include *Caenorhabditis elegans* HEN-1 (ref. 24) and MIG-13 (ref. 25), and the mammalian proteins 8D6 (ref. 26) and scospondin[27]. Jeb is the first among these to have an identified signalling receptor and a defined biological pathway. We anticipate that this discovery will lead to the identification of receptors and modes of action for other members of this class of signalling molecule.

The extracellular portions of mammalian and *Drosophila* Alk have common domain architectures. Their respective ligands are therefore also likely to share structural features. However, two closely related cytokines that are structurally unrelated to Jeb, pleiotrophin and midkine, have been identified by phage display as potential high-affinity ligands for human ALK[28]. In *Drosophila*

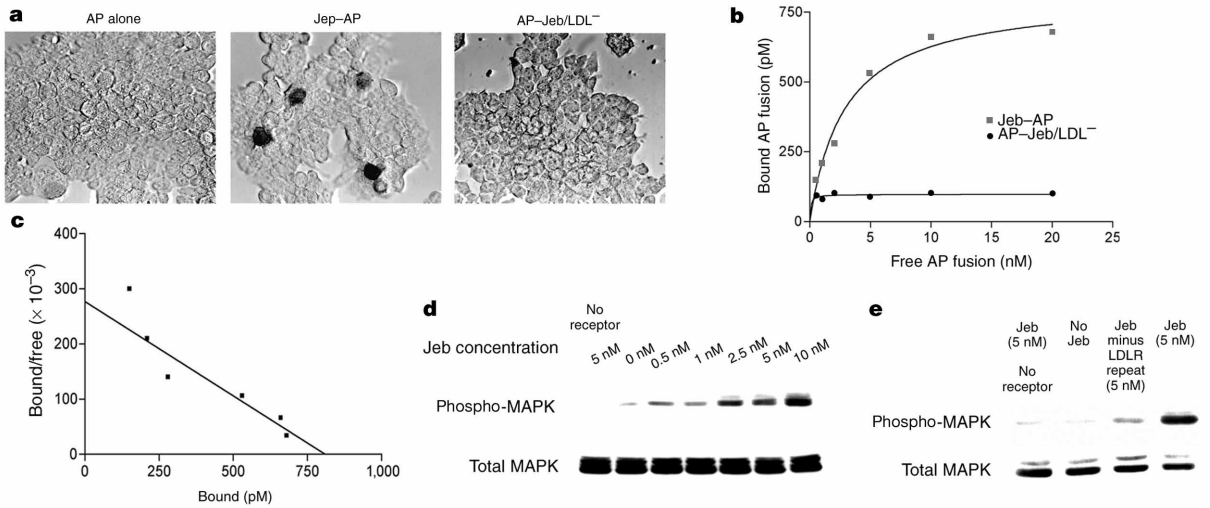

Figure 4 Jeb specifically binds with high affinity to *Drosophila* Alk and activates MAPK in mammalian tissue culture cells. **a**, Qualitative binding assays of Jeb–AP fusion protein to human 293T cells transfected with full-length *Alk* cDNA. Binding of AP is visualized by dark precipitates in transfected cells (middle panel). Cell-associated AP activity depends on Jeb and the type-A LDL receptor repeat in Jeb (right and left panels). **b**, Jeb binding to *Alk*-transfected 293T cells is saturable over a narrow concentration range and shows a K_d of 2.2 nM. *Alk*-transfected 293T cells were incubated with various concentrations of Jeb–AP and the retained AP activity was determined by quantitative colorimetric assays. As in the qualitative binding assays, specific high-affinity binding is dependent on the type-A LDL receptor repeat in Jeb. **c**, Scatchard analysis demonstrates a single class of high-affinity binding sites on *Alk*-transfected 293T cells with a K_d of 2.2 nM. **d**, Increased concentration of Jeb–AP increases phospho-MAPK with half-maximal activation occurring at about 2 nM. **e**, Activation of the Ras/MAPK cascade, similar to binding, requires the type-A LDL receptor repeat in Jeb. Protein loading controls are shown as an immunoblot for total MAPK protein (**d**, **e**, bottom).

two clustered genes, *miple1* and *miple2*, encode polypeptides related to midkine/pleiotrophin. Similar to the mammalian genes, *Drosophila miple1* and *miple2* are expressed widely during embryogenesis (J.B.W., unpublished data). So, unlike Jeb, Miple1 and Miple2 cannot control the spatially restricted activation of Alk in the visceral mesoderm, although they may have an auxiliary function in Alk activation. The potential functions of Jeb-related molecules in mammalian Alk activation and the possible contribution of midkine/pleiotrophin-related factors to Alk signalling in *Drosophila* can now be tested by genetic and molecular approaches. The characterization of the Jeb/Alk signalling pathway in *Drosophila* is also likely to enhance our understanding of vertebrate Alk signalling in development and cancer. As most studies of mammalian Alk have focused on the role of oncogenic versions in cellular transformation, our current understanding of Alk's normal function in mammals is rudimentary (reviewed in ref. 2). In light of the known conservation of genetic pathways in the cardiac and splanchnic mesoderm[8,29], our insights into the regulation of *org-1* expression in *Drosophila* are potentially relevant for the understanding of the regulation of human *TBX1* and its roles in congenital cardiovascular and craniofacial disease. In addition, the specific expression of *Drosophila* and mouse Alk in the central nervous system[2,21] suggests a conserved role of Alk signals in the development or function of neuronal tissues. □

Methods

Fly strains

We used the following *Drosophila* lines: *jeb* $^{-c}$ (ref. 1), UAS-*jeb* (ref. 1), *Df(2R)Vg89e88* (an uninverted insertional transposition, *Tp(2;3)Vg89e88*, with a haplolethal deficiency component in the interval 52B3-C1; 53E2-F1), UAS-*Ras85D*^{V12}, UAS-*hRaf*, 2 × PE *twi-Gal4* (from the Bloomington Stock Collection), *rP298* (from A. Nose), *bap3-lacZ*, *bap3-Gal4* (ref. 8), UAS-*Alk*^{act}, UAS-*Alk*^{DN}, *bap3-Alk* (this study). Rescue of visceral mesodermal *Df(2R)Vg89e88* phenotypes was accomplished by recombining *bap3-Alk* onto the *Df(2R)Vg89e88* chromosome.

Plasmid constructions

The construct for constitutively active Alk (Alk^{act}) was made by fusing codons 1–117 of human nucleophosmin (*NPM*)[22] to codons 1129–1701 of *Drosophila Alk*, cloned into pUAST to produce UAS-*Alk*^{act} transgenic lines. The dominant negative Alk (Alk^{DN}) construct, also in pUAST, encodes the entire extracellular domain, transmembrane domain and a short tail of the intracellular domain (amino acids 1–1143) of Alk. The rescue construct, *bap3-Alk*, uses a *bap* enhancer element, *bap3* (ref. 8), and the *hsp70* basal promoter to drive the expression of *Alk* complementary DNA fused to an *SV40* 3′ untranslated region. For details see Supplementary Information.

RNA interference and embryo staining

RNA interference was performed as described previously[30]. *Alk* dsRNA (nucleotides 978–2089; 7.5 μM) was injected posteriorly into pre-blastoderm embryos from *bap3-lacZ* or *rP298* flies. Antibody and antibody/*in situ* hybridization double-fluorescent staining was performed as described previously[3,8] (see Supplementary Information for antibodies and probes used).

AP fusion protein production

Full-length and truncated open reading frames of *jeb* were subcloned in frame with the APtag-4 expression vector (GenHunter). 293T cells, cultured with DMEM plus 10% serum in 100 mm plates, were transiently transfected for 24 h with the fusion constructs using Lipofectamine (Invitrogen). After 6–8 days of growth in Opti-MEM (Gibco) containing 0.3% serum or DMEM containing 0.3% serum, conditioned medium was concentrated by ultrafiltration. Stability of intact fusion protein was confirmed by immunoblotting using an anti-alkaline phosphatase antibody (GenHunter). The concentration of fusion protein was determined by measuring AP activity at 405 nm using a plate reader as described previously[23].

Cell staining and quantitative binding

293T cells were cultured on plates pre-coated with poly-D-lysine and transiently transfected for 24 h with Flag epitope-tagged Alk cDNA subcloned into the pCDNA3.1 expression vector using Lipofectamine. Binding of AP and AP fusions and staining was performed as described previously[23]. For quantitative assays, 293T cells were transiently transfected for 24 h with pCDNA3.1-Alk. Cells were treated with a range of concentrations of Jeb–AP or AP–Jeb/LDL$^-$ diluted in HBAH buffer (Hanks Balanced Salt Solution, BSA (0.5 mg ml^{-1}), 0.1% (w/v) NaN_3, 20 mM HEPES, pH 7.0) and binding to the cell surface was determined as described previously[23] using a plate reader to measure the change of absorbance (A) at 405 nm. After converting A to the quantity of protein bound, the binding curve and K_d were obtained using Prism3 software. Equivalent levels of full-length receptor expression in transfected cells were demonstrated by immunoblotting using anti-Flag M2 antibody (Sigma).

MAPK phosphorylation

After transfection of 293T cells as above, cells were serum-starved for 24 h in DMEM plus 0.3% serum, treated with 5 nM Jeb–AP or AP–Jeb/LDL$^-$ produced in DMEM plus 0.3% serum for 20 min at 37 °C, placed on ice and washed twice with PBS. Cells were lysed with 0.5 ml lysis buffer (20 mM HEPES pH 7.5, 10 mM EGTA, pH 8, 1% NP-40, 2.5 mM $MgCl_2$, 200 mM sodium ortho-vanadate, 1 nM okadaic acid, protease inhibitor cocktail tablet

(Roche)) on ice. After clearing of the lysates by centrifugation, total and phosphorylated MAPK content was monitored by immunoblotting using p44/42 MAPK and phospho-p44/42 MAPK (Thr 202/Tyr 204) antibodies (New England Biolabs).

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Correspondence and requests for materials should be addressed to J.B.W. (weissjo@ohsu.edu) or M.F. (manfred.frasch@mssm.edu).

Jeb signals through the Alk receptor tyrosine kinase to drive visceral muscle fusion

Camilla Englund[1]*, Christina E. Lorén[1]*, Caroline Grabbe[1], Gaurav K. Varshney[1], Fabienne Deleuil[1,2], Bengt Hallberg[2] & Ruth H. Palmer[1]

[1]*Umeå Center for Molecular Pathogenesis,* [2]*Department of Medical Biosciences, Umeå University, Umeå, S-901 87, Sweden*

* These authors contributed equally to this work

The *Drosophila melanogaster* gene *Anaplastic lymphoma kinase* (*Alk*) is homologous to mammalian Alk, a member of the Alk/Ltk family of receptor tyrosine kinases (RTKs)[1]. We have previously shown that the *Drosophila* Alk RTK is crucial for visceral mesoderm development during early embryogenesis[2]. Notably, observed *Alk* visceral mesoderm defects are highly reminiscent of the phenotype reported for the secreted molecule Jelly belly (Jeb)[3]. Here we show that *Drosophila* Alk is the receptor for Jeb in the developing visceral mesoderm, and that Jeb binding stimulates an Alk-driven, extracellular signal-regulated kinase-mediated signalling pathway, which results in the expression of the downstream gene *duf* (also known as *kirre*)[4,5]—needed for muscle fusion. This new signal transduction pathway drives specification of the muscle founder cells, and the regulation of Duf expression by the *Drosophila* Alk RTK explains the visceral-mesoderm-specific muscle fusion defects observed in both *Alk* and *jeb* mutant animals.

Alk was first described in non-Hodgkin's lymphoma[6,7], and is now known to be involved in many genetic translocation events[8,9]. Alk function in higher vertebrates has remained elusive, despite identification of mouse and human homologues[10,11]. *Drosophila* Alk is expressed in the developing visceral mesoderm during embryogenesis[1], and *Alk* mutants reveal that no functional midgut is formed[2]. The visceral musculature in *Drosophila* is syncytial[12,13], and arises during embryogenesis through the fusion of multiple myoblasts[14,15]. The process of myoblast fusion in *Drosophila* is a dynamic relationship between two myoblast types: the 'founder cells' and the 'fusion-competent myoblasts'[16]. Progenitor myoblasts give rise to founder cells that serve as 'seeds' for muscle formation, which the fusion-competent myoblasts then recognize and fuse with[16,17].

Mutant *Drosophila Alk* and *jeb* larvae do not ingest food (Fig. 1; compare b, c with a) and lack discernible intestinal structures, whereas heterozygous siblings are robust with healthy appetites. Therefore, embryonic visceral mesoderm development was further analysed using fasciclin III, which is a marker for differentiated visceral mesoderm. In wild-type embryos Alk and fasciclin III expression patterns overlap as the midgut takes form (Fig. 1d, g; j, m in detail). Alk mutant protein is visible in *Alk* mutants because our Alk antibodies are raised to an amino-terminal epitope[2]. In *Alk* mutant embryos the visceral mesoderm is observed as a disorganized group of Alk-positive cells at stage 13 (Fig. 1; compare e with d; panel o in detail)[2]. A similar phenotype is observed in *jeb* mutants, with Alk-positive visceral mesoderm cells scattered in a disorganized manner instead of the organized band of cells normally observed at this stage (Fig. 1; compare f with d; panel n in detail). The earliest developmental stage at which a mutant phenotype in the visceral mesoderm can clearly be observed in *jeb* and *Alk* mutants is at stage 11 (Fig. 1j–l). In wild type the muscle founder cells are specified in the visceral mesoderm, and become arranged in an organized column of cells located ventrally (Fig. 1j,

